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From the resolution revolution to evolution: structural insights into the evolutionary relationships between vesicle coats and the nuclear pore

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Nuclear pores and coated vesicles are elaborate multi-component protein complexes that oligomerize on membranes, and stabilize or induce membrane curvature. Their components, nucleoporins and coat proteins, respectively, share similar structural folds and some principles of how they interact with membranes. The protocoatomer hypothesis postulates that this is due to divergent evolution from a common ancestor. It therefore has been suggested that nucleoporins and coat proteins have similar higher order architectures. Here, we review recent work that relied on technical advances in cryo-electron microscopy and integrative structural biology to take a fresh look on how these proteins form membrane coats *in situ*. We discuss the relationship between the architectures of nuclear pores and coated vesicles, and their evolutionary origins.

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Introduction

The origin of eukaryotes is one of the greatest mysteries in biology. One hallmark of eukaryotes is compartmentalization into organelles. Several theories have been proposed about the sequence of evolutionary events that led to eukaryotic compartmentalization [1,2••,3]. Mitochondria and chloroplasts originated from the internalization of bacterial endosymbionts [1]. However, how exactly the elaborate endomembrane system including the nucleus, the endoplasmic reticulum (ER), and the Golgi apparatus has evolved, remains enigmatic. Our ability to reconstruct the exact order of evolutionary events based on morphological comparison or sequence analysis is limited because no eukaryotes have been identified yet that diverged before the specialization of nucleus, ER and Golgi apparatus.

Several different membrane coats have co-evolved with the nuclear envelope (NE), ER and Golgi apparatus and facilitate trafficking between these compartments and the cell exterior. The clathrin coat facilitates endocytosis, that is the uptake of extracellular material, and takes part in other cargo sorting pathways [4,5]. The coat protein complex (COP) I mediates retrograde transport from the Golgi to the ER or bidirectional transport within the Golgi [4,5]. COP II is required for anterograde transport from ER to Golgi and exocytosis [5]. All these three membrane coats facilitate convex membrane curvature. Nuclear pore complexes (NPCs) do not form vesicle coats but instead fuse the inner and outer nuclear membranes to form channels across the nuclear envelope through which nucleocytoplasmic trafficking occurs [6]. These channels have both convex and concave membrane features.

The comparison of these different membrane coats might shed light on the order in which the compartments originated. However, the sequence similarity between proteins associated with different compartments is relatively low, making it extremely difficult to understand their ancestry. Nevertheless, several clues about their evolution can be derived through analysis of protein structures. Structures obtained using X-ray crystallography and homology modeling showed that coat proteins are composed of recurring β-propeller and α-solenoid domains. This observation led to formulation of so-called 'protocoatomer' hypothesis [7••,8]. It postulates that clathrin, COPI, COPII and NPC coats are derived from a common ancestral membrane-curving module. This notion is underlined by sequence and structural similarity between subunits of these coats [9,10] as well as proteins moonlighting between the different membrane coats, such as Sec13. It has further been predicted that clathrin, COPI, COPII and the NPC might oligomerize into similar lattice-like coats *in situ* [10–12]. However, until relatively recently it remained very challenging to verify how these proteins interact with each other *in situ* in the context of membrane. Particularly in case of COPI and

the NPC, X-ray crystallographic analysis had revealed a considerable number of protein interfaces with unclear role for the higher order arrangement formed by multiple protomers *in situ*. Although electron microscopy had been used to visualize nuclear pores and the respective coats, the resolution remained insufficient for placing high-resolution structures into context. It was however clear that understanding the similarities between the coats may give hints regarding their evolutionary path that led to vesicle trafficking systems and respective compartments.

Recent technological developments in cryo-electron microscopy (cryo-EM) significantly facilitated addressing this challenge. The so-called 'resolution revolution' [13] has been fueled by both hardware and software innovations, such as more stable specimen stages or routines for automated high-throughput data acquisition [13]. A key breakthrough was the development of highly sensitive direct electron detectors (DEDs) producing micrographs with considerably better signal-to-noise ratio. The benefits of those developments for single particle analysis and *in vitro* structural biology have been often discussed [14]. However, also cryo-electron tomography (cryo-ET) with subsequent subtomogram averaging, a technology that is very well suited for *in situ* studies of membrane associated complexes [15], experienced a leap forward. This led to profound insights [16••,17,18••,19–21] and in many cases facilitated the placement of high-resolution structures into the *in situ* context for the very first time. Here we review these studies together with work on high-resolution structures to reconcile similarity between the nuclear pores and vesicle coats. We discuss implications for the structural and evolutionary relationships between both and potential evolutionary trajectories.

Molecular architecture of membrane coats

The clathrin coat is susceptible to biochemical enrichment and had been characterized *in vitro* already before the resolution revolution. Electron microscopy studies demonstrated that clathrin-coated vesicles are composed of the inner and outer coats [22]. The inner coat is composed of heterotetrameric adaptor complexes. The outer coat is formed by trimers of clathrin heavy and light chains, called triskelions, which oligomerize to form a polygonal lattice [23,24] (Figure 1a). The heavy chain contains an N-terminal β-propeller domain [25], which binds to the inner coat, and the C-terminal α-solenoid 'leg' domain, which trimerizes to form the vertex of the triskelion. This β-propeller-α-solenoid domain architecture is a recurrent and very prominent feature of membrane coats.

Similar to clathrin, COPII vesicles are also built from distinct inner and outer coats [26]. The inner coat is made up of membrane and cargo binding Sec23/24-Sar1 adaptor complex, which is however structurally different to COPI and clathrin adaptins. The outer coat is composed of dimers of Sec13 and Sec31 [27]. The 12 Å resolution cryo-EM structure of the outer coat [28] revealed that the outer coat forms a polyhedral lattice. In this lattice, every edge comprises two copies of Sec13 and Sec31 respectively and each vertex of the cage is formed by direct interactions of four copies of Sec31 β-propellers (Figure 1c).

Based on the similarities between COPI and clathrin subunits it could have been expected that COPI is also composed of an outer and inner coat. In fact, some COPI subunits are homologs to adaptins that form the inner coat of clathrin. Moreover, the α and β' subunits of COPI also consist of N-terminal β-propellers and a C-terminal α-solenoid. The crystal structure of the β'-COPI in complex with α-COPI α-solenoid domain revealed that the α-solenoids can dimerize. Intriguingly, in the crystal structure, three copies of the α and β' dimer formed a triskelion and were interpreted as lattice contacts forming the outer coat, in analogy to clathrin [11]. However, recent cryo-ET analysis of reconstituted COPI vesicles, facilitated by new data acquisition schemes and with the use of DEDs, revealed a very different architecture [16••,19,29•,30•] (Figure 1b). First, in the COPI coat, α and β'-COPI do not arrange into a triskelion, revealing that the COPI triskelion-like interaction is possibly a crystallographic artefact. Second, the subunits do not arrange into the outer and inner coat. Instead, they form a single layer on a membrane. Last, the α and β'-COPI do not assemble in a continuous lattice but together with the other subunits form repeating triads connected by flexible linking domains.

The NPC coat is formed by two major scaffold nucleoporin (Nup) complexes that are spatially separated (Figure 1d). The so-called inner ring complex interacts directly with the fused inner and outer nuclear membranes, while the Y-complex forms the two outer rings in the cytoplasmic and nuclear compartments that distally connect to the inner ring. The consecutive β-propeller-α-solenoid arrangement that is typical to the aforementioned membrane coats is recurrent in scaffold Nups. Thus, a lattice model in which the β-propeller domains of scaffolding Nups interact to form a lattice-like coat had been inspired by earlier models of vesicle coats [31,32]. However, *in situ* structural analysis of human NPC by cryo-ET together with integrative structural modeling revealed a different picture [6,17,18••,33••]. Although the β-propellers of the Y-complex are important interaction motifs, they are not arranged into a triskelion cage. Instead, 32 Y-complexes arrange into two reticulated outer rings [34]. The inner ring also consists of 32 protomers and shares a similar repertoire of structural folds with the outer rings [18••,33••]. NPCs of other species also exhibit ring architecture but contain varying number of rings. Recent stoichiometric measurements of yeast NPCs [35,36] revealed only 16 Y-complexes arranged in